THE PREPARATION OF CRYSTALLINE LACTIC ACID

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On account of its importance in intermediary metabolism, lactic acid was among the first compounds chosen in our plan, which we have described in a previous communication (1), to augment the available data on the free energies of formation of substances significant in biological chemistry. It was necessary for this purpose to obtain pure crystalline lactic acid, free of water, anhydride, and lactide. The only description in the literature of the preparation of crystalline lactic acid is that of Krafft and Dÿes (2). Table I shows that the product obtained by their method contains relatively large quantities of anhydro impurities. The subject of the present communication is the description of a method which yields the active isomers of lactic acid in a crystalline state, free of water, anhydride, and lactide, supplemented by the description of two methods of separating the active forms from the commercial syrup.[1] Lactic acid commercially available at present either is in the form of the U.S.P. syrup, which usually exhibits a low optical activity corresponding to the excess it happens to contain, which is variable, of one or the other optical isomer, or is the expensive zinc sarcolactate. The methods described below now make it possible to obtain easily and quickly and at low cost large quantities of both active isomers in a relatively high degree of purity.

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[1] In this communication the form of lactic acid commonly named sarcolactic or *d*-lactic acid is designated as *l*(+)- or *l*-lactic acid. The salts of this form are levo- and the free acid dextrorotatory. The opposite form is correspondingly designated as *d*(−)- or *d*-lactic acid. Optically inactive lactic acid is referred to as the *dl* form without any implication regarding its constitution; *i.e.*, whether it is a simple mixture of equal quantities of the two active forms, or a definite compound.

The summary in Table III of some of the physical properties of the free acids prepared by these methods shows that they probably have not been obtained in as pure a state out of solution hitherto. For example, the melting points of the active forms are more than 25° higher than the values given in standard reference works (3, 4). They are also less hygroscopic than they are commonly described.

We shall describe first the preparation of the crystalline free acids from the commercial syrup (which contains as a rule about 50 per cent lactic acid, 30 per cent anhydride and lactide, and 15 per cent water). Briefly, the method consists of fractional distillation followed by fractional crystallization from a mixture of equal volumes of ethyl and isopropyl ethers. The typical procedure was as follows: 200 cc. of syrup were distilled from a 1 liter Claisen flask, first with a water pump at about 60° until most of the water had been driven off, and then, with the outside bath temperature raised to 105°, with an oil pump through two liquid air traps at a pressure of about 0.1 mm. A middle fraction of about 75 cc. was collected and redistilled with the oil pump. The middle fraction from the second distillation, usually about 60 cc., was then set away in ice-salt to crystallize. Even when a large quantity of lactic acid is required, it is preferable to carry out the distillation a number of times with small portions because with large quantities greater losses are incurred during the longer heating required at 105°.

In order to minimize anhydride formation in the syrup and in the vapor through overheating, the side arm of the distilling flask, internal diameter about 2 cm., was fused into the neck about 1 cm. above the bulb and was shielded from the heat of the paraffin bath by asbestos board. For the same reason the filter flask in which the distillate was received was kept at room temperature in a water bath during the collection of the middle fractions.

In about 2 hours the first nuclei of crystals appeared in the second middle fraction set away in ice-salt. Removed then to the temperature of ice and shaken vigorously, the syrup soon became a solid crystalline mass. The product at this stage corresponds to that obtained by Krafft and Dÿes. The degree of anhydro impurity present is shown in Table I.

The crystalline lactic acid was now dissolved in an equal volume of a mixture of equal parts of ethyl and isopropyl ethers (dried over

sodium), complete solution requiring about ½ hour at 37°. After some hours at the temperature of ice-salt, crystallization set in. After another hour, when the contents had become a nearly solid

TABLE I

Titration of Twice Distilled Lactic Acid (Krafft and Dyes (2))

Weight	First titration, N/14 NaOH	Back titration, N/14 NaOH	Total titration, N/14 NaOH	Calculated value for pure lactic acid, N/14 NaOH	Free lactic acid calculated on basis of sole impurity as	
					Anhydride	Lactide
gm.	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*
1.0447	162.0	1.5	163.5	162.4	93.7	97.2
1.0260	159.2	1.4	160.6	159.5	93.7	97.2
1.0513	163.0	1.5	164.5	163.5	93.7	97.2

TABLE II

Titration of Crystalline Lactic Acid

Specimen	Weight	First titration, N/14 NaOH	Back titration, N/14 NaOH	Total titration, N/14 NaOH	Calculated titration value for pure lactic acid, N/14 NaOH
	gm.	*cc.*	*cc.*	*cc.*	*cc.*
l(+)-Lactic acid prepared by recrystallization of distillate three times from the mixed ethers	0.5004	77.84	0.06	77.90	77.86
	0.6836	106.30	0.04	106.34	106.37
	0.7083	110.09	0.04	110.13	110.21
Same after melting and heating to 56° and crystallizing again	0.9963	154.62	0.16	154.78	154.87
	0.7343	114.10	0.28	114.38	114.15
l(+)-Lactic acid from zinc ammonium salt, recrystallized three times from the mixed ethers	0.4182	65.06	0.05	65.11	65.00
	0.4319	67.05	0.05	67.10	67.08
d(−)-Lactic acid from zinc ammonium salt, recrystallized three times from the mixed ethers	0.5498	85.40	0.00	85.40	85.46
	0.3990	61.95	0.00	61.95	62.02

mass of crystals, they were filtered quickly with suction. The crystallization was repeated three times. After the first crystallization, crystallization began in the subsequent ethereal solutions

immediately on cooling under the tap. After the final recrystallization, the fine white crystals were dried at room temperature in a vacuum desiccator. Titration of this product showed that it contained less than 0.1 per cent of impurities such as water, anhydride, or lactide (Table II).

The fractional crystallization from the mixed ethers effects also a separation of the active isomer, which was in excess in the original syrup (here the *l*(+) form), from the main bulk of inactive material. This was shown by the optical activity of the ethereal mother liquor, which was nearly zero (indicating that no resolution, but only a separation of the excess of active isomer from the inactive form, had occurred), and by the melting point of a mixture of the *l*(+) acid obtained by this method and some *l*(+) acid prepared from the zinc ammonium *l*(+) salt. Separately the melting point of each form was 52.7–52.8°. The mixed melting point was 52.7°. On the other hand, the melting point of an equimolar mixture of the acid obtained by fractional crystallization of the distillate from the crude syrup with that prepared from zinc ammonium *d*(−)-lactate was 16.8° (Table III).

We have not had occasion as yet to try the separation by this method of the *d*(−) acid from a preponderance of the *dl* form because all the commercial syrups we secured contained an excess of the *l*(+) isomer. Nevertheless, we feel confident in recommending the method because the solubility in the mixed ethers of the two active acids prepared from their respective zinc ammonium salts was the same, both being much lower than that of the *dl* form. *dl*-Lactic acid can also be crystallized from the mixed ethers, but the temperature of solid CO_2 is necessary for a good yield, while for the active acids 0° is sufficiently low.

The active acids were also prepared by way of their zinc ammonium salts obtained by resolution of the commercial syrup. The method of resolution employed was essentially that described by Purdie and Walker (5). 1000 cc. of lactic acid syrup were made alkaline to litmus with concentrated ammonia, and then boiled until acid again. This was repeated several times until long boiling was necessary to restore a faint acidity. 560 gm. of zinc *dl*-lactate (air-dried) were now added. The solution was again boiled until all the salt had dissolved, and while still boiling the volume was made up to 2 liters. It was filtered rapidly with suction while

still hot, and then transferred to a clean and dry 4 liter beaker. The beaker was placed in a bath of ice water and stirred. When cool it was seeded with about 0.1 gm. of finely powdered zinc ammonium *l*(+)-lactate. Within a short time after the seed had been thoroughly stirred in, the crystals of the optically active zinc ammonium salt began to cloud the solution. The process was controlled by microscopical examination of the crystals from time to time. The optically active double salt forms short, relatively wide, single, rectangular or square prisms; the inactive salt forms long narrow rods frequently arranged in radiating clusters. The inactive form appeared when the solution was too dilute, or when it was set away at 0–2°. When the syrup was too concentrated, crystallization was very slow, and after 24 hours only a poor yield of active crystals contaminated with the inactive form was obtained. If the solution was too dilute, it was boiled again until a definite quantity of water (determined by weight) was driven off; if too concentrated, it was also boiled, then brought to a definite weight with water, and, after cooling, seeded again. When the conditions were suitable, an abundant quantity of optically active crystals appeared in an hour throughout the solution. The beaker was left to stand overnight at room temperature (15–20°). When microscopical examination showed the copious deposit at the bottom of the beaker to be not more than slightly contaminated with inactive crystals, the supernatant liquor was decanted off and centrifuged. The precipitates in the centrifuge cups and in the beaker were washed three times with 95 per cent alcohol, and, after drying in air, the optical activity of an 8 per cent aqueous solution was measured. The amount of solid remaining undissolved in making the 8 per cent solution was a good measure of the degree of contamination with inactive salt.

After the first batch of active crystals had been separated from the syrup, there was dissolved in it a quantity of zinc *dl*-lactate, equal to the weight of the active double salt obtained, and then it was seeded with the opposite active form. Purdie and Walker and others have reported obtaining large quantities of both active forms by this method of repeated crystallization after seeding alternately with one and then the other active form. In spite of many attempts this method always failed us. Repeatedly on seeding with zinc ammonium *d*(−)-lactate we obtained either the *dl*

form, or large quantities of the $l(+)$ double salt. From one batch of syrup in three successive crystallizations we obtained over a kilo of the $l(+)$ double salt, although the last two seedings were with $d(-)$ double salt. The probable reason for our failure is that the syrup contained too great an excess of the $l(+)$ isomer. We did succeed in obtaining a small quantity of the $d(-)$ double salt each time on seeding a virgin syrup with $d(-)$ seed, although it contained an excess of the $l(+)$ form.

We found also that when zinc ammonium $d(-)$-lactate was mixed with a large quantity of the double salt of the *dl* acid, the active salt could be separated out by warming to 55° for about an hour and then setting aside at room temperature overnight. We did not succeed in obtaining the double salt of the $l(+)$ acid by this method, probably because of the excess of the $d(-)$ in the crude syrup. This experience suggests that large yields of either form of active zinc ammonium lactate (3 to 4 times that separated by Purdie and Walker) can be obtained by their method of seeding a supersaturated solution if the initial lactic acid syrup contains an excess of the same active form as the seed added. The opposite form can also be obtained by seeding a virgin syrup, as in our case, but the yields are small. If the crude syrup contains a 20 per cent or greater excess of one active form, the preferable method for obtaining a large quantity of the pure isomer, which is in excess, is distillation and crystallization from the mixed ethers as described above, without previous precipitation of the active zinc ammonium salt.

The $l(+)$ seed was prepared as follows: A quantity of zinc $l(+)$-lactate was converted to the ammonium salt by treatment with H_2S, followed by ammonia, and was then added to twice the equivalent quantity of lactate in the form of the zinc salt. The combined solutions were concentrated on the water bath to a syrup, and then cooled. The short rectangular prisms of the active double salt obtained were separated from the syrup by suction filtration, washed with 95 per cent alcohol, and air-dried. The $d(-)$ seed was obtained by means of the morphine salt by Patterson and Forsyth's modification (6) of the method of Irvine (7).

The following was the typical method of preparation of the active acid from its double salt. 500 gm. of zinc ammonium $d(-)$-lactate were dissolved in 1200 cc. of cold water and filtered imme-

diately. The clear filtrate was set away overnight at about 2°. The next morning the precipitate of zinc lactate was separated by suction filtration and washed with distilled water until the washings no longer gave a positive test with Nessler's reagent. The salt was air-dried to constant weight and its water of crystallization determined by heating at 100° to constant weight. The theoretical value for the optically active form is 12.9 per cent, corresponding to 2 molecules of water of crystallization. The *dl* form contains 3 molecules of water of crystallization. When the water of crystallization was greater than 12.9 per cent, the salt was recrystallized until the theoretical value was obtained. If the zinc salt was pure from the outset, the collected filtrate and washings of ammonium lactate were boiled with $Ca(OH)_2$ to drive off the ammonia, and the free acid was obtained by precipitating the calcium with oxalic or sulfuric acid. If the zinc salt was impure, this free acid was converted to the zinc salt by boiling with zinc oxide, and then recrystallized until the theoretical percentage of water of crystallization was obtained. The free acid was liberated from the zinc salts by treatment with H_2S.[2] The combined aqueous solutions of the free acid were now concentrated at 60° with a water pump to a syrup, then distilled, and crystallized from the mixture of ethyl and isopropyl ethers as described above. In this manner about 120 gm. of free acid were obtained from about 500 gm. of each active double salt.

The purity of the free acids prepared, *i.e.* their freedom from anhydride, lactide, and water, was determined by titration with N/14 NaOH and phenolphthalein as indicator. The following standardized technique was employed. Between 0.4 and 1.0 gm. of the acid was transferred quickly to a tared weighing bottle, which was then covered, weighed, and inserted with tongs into a wide necked flask containing 100 cc. of CO_2-free distilled water.

[2] Free lactic acid is adsorbed in large quantities by such precipitates as zinc sulfide, calcium sulfate, and calcium oxalate, and boiling several times with large quantities of distilled water was necessary in order to avoid large losses. The precipitates were washed until the washings gave a negative reaction in the following test described by Denigès (8). 0.2 cc. of solution and 2 cc. of concentrated H_2SO_4 are heated in a boiling water bath for 2 minutes. After cooling under the tap, a drop of an alcoholic solution of guaiacol is added. A fuchsin red color develops with 0.01 mg. of lactic acid.

The stopper was then shaken off the weighing bottle, and the titration begun with a stream of CO_2-free nitrogen bubbling continuously through the solution. When the apparent end-point was reached, the weighing bottle and lid were removed with tongs and rinsed with CO_2-free water, the rinsings being collected in the flask containing the lactic acid. The solution was now brought to a boil and again titrated to the first appearance of pink. The total alkali added to this point was designated as the first titration value. 5 cc. of N/14 NaOH were now added and the solution again boiled for 3 minutes, after which the remaining excess of alkali was back titrated with N/14 HCl. The addition of excess alkali, boiling, and back titration were repeated until the difference between the acid used and the alkali added in one back titration was not more than 0.2 cc. The difference between the total excess alkali added and the amount of N/14 HCl used in the back titration was noted as the back titration value. If lactide and anhydride are present (in the absence of a significant amount of water), the sum of the initial titration figure and the total acid liberated by boiling in excess of alkali is greater than the titration figure calculated on the assumption that the lactic acid weighed out was free of water, anhydride, or lactide. If T cc. of N/14 NaOH is the total titration figure observed per gm. of material weighed out, the percentage of free lactic acid is given by the formula $\frac{172.7 - T}{17.3} \times 100$, on the assumption that the only impurity is anhydride, and by the formula $\frac{194.4 - T}{39} \times 100$ if the impurity is assumed to be solely lactide. Typical titration results with pure and impure lactic acids are given in Tables I and II.

Table III summarizes the melting points, dissociation constants, and hygroscopic properties of the pure acids. The melting point determinations were made on 10 to 12 gm. samples in a wide test-tube stirred constantly with dry nitrogen. The inside temperature was read with an Anschütz thermometer. The temperature of the water bath outside was kept about 1° higher than the inside during the melting, and 1° lower during the subsequent crystallization. The temperatures are the equilibrium temperatures observed during the melting when both phases were present. The crystallization temperatures were 1° lower. The melting points

of the pure active forms observed are much higher than the values of 25–27° given in Landolt-Börnstein (3) or the "International critical tables" (4), which are based on the work of Jungfleisch and Godchot (9). The low values found by these authors are probably to be attributed to incomplete resolution. This surmise is supported not only by the difference in the melting points, but also by the failure of other workers (7) and ourselves to obtain a satisfactory resolution by the method described by Jungfleisch and Godchot. The melting point of *dl*-lactic acid prepared by melting together and then crystallizing equal quantities of the

TABLE III

Summary of Some of the Properties of the Optically Active Lactic Acids

Specimen	Melting point	Water absorbed from the air at room temperature; fraction of original weight	Dissociation constant at 25°
	°C.		*pK*
d(−)-Lactic acid from recrystallization of distillate (Specimen A)	52.7	0 in 4 hrs.; 2% in 20 hrs.	3.81 ± 0.01
d(−)-Lactic acid from zinc ammonium salt (Specimen B)	52.8	0 in 4 hrs.; 2% in 20 hrs.	3.83 ± 0.01
2 parts Specimen A + 1 part Specimen B	52.7		
l(+)-Lactic acid from zinc ammonium salt	52.8	1% in 4 hrs.; 3% in 20 hrs.	3.79 ± 0.01
Equal weights of Specimen A and *l*(+)-lactic acid	16.8	In liquid state 3% in 3 hrs.; 10% in 15 hrs.	3.81 ± 0.01

pure *l*(+) and *d*(−) acids was found to be 16.8°, nearly 1° lower than the melting point given by Krafft and Dÿes for their *dl*-lactic acid. The probable explanation for this difference is that, apart from contamination with water or anhydro compounds, the product obtained by Krafft and Dÿes was a mixture of the *dl* and one of the active forms. Such mixtures we have found melt at temperatures higher than 16.8°, according to the degree of excess of one of the active forms. The general experience has been that commercial lactic acid syrup, from which Krafft and Dÿes obtained their product, contains nearly always an excess of one of

the active forms; and this mixed composition is carried over into the distillates.

The dissociation constants were determined in the usual manner by electrometric titration with Moloney hydrogen electrodes (10) in duplicate, against a saturated calomel half-cell. In the computation of the dissociation constants we have assumed the activity coefficient of the undissociated acid to be 1, which is justified by the freezing point data given in the "International critical tables." The activity of the lactate ions was estimated by the simplified form of the Debye-Hückel equation. The values obtained, there-

TABLE IV

Optical Rotations of Lactic Acid and Its Salts When $\lambda = 5461$ *Å., at 21–22°*

	Concentration	α	$[\alpha]_{Hg}^{21-22}$	$[M]_{Hg}^{21-22}$
	gm. per cent	*degrees*		
d(−)-Lactic acid	8.00	+0.41	+2.6	+2.3
" "	4.00	+0.18	+2.3	+2.1
l(+)-Lactic "	8.00	−0.41	−2.6	−2.3
" "	4.00	−0.16	−2.0	−1.8
Zinc ammonium *l*(+)-lactate	8.00	−1.10	−6.9	−8.9
" " *d*(−)-lactate	8.00	+1.10	+6.9	+8.9
Sodium *l*(+)-lactate	4.20	−1.15	−13.7	−15.8
" *d*(−)-lactate	7.05	+1.70	+12.1	+13.5

fore, approximate thermodynamic dissociation constants, pK (as distinguished from titration constants, usually designated as pK′).

The avidity of the free acids for water was determined approximately by exposing a weighed quantity of the acid to the air at room temperature in an open weighing bottle. Table III shows that the pure optically active acids are only slightly hygroscopic. This also runs counter to the description usually given, which is derived from the papers of Jungfleisch and Godchot. As in the case of the difference in the melting points, the discrepancy probably is to be ascribed to incomplete resolution, and possibly also to contamination of the product obtained by these authors by water and anhydride, since the free acids were prepared from their

quinine salts by the distillation method of Krafft and Dÿes, and were not purified further.

The pure optically active acids are relatively quite stable. Kept in a desiccator at room temperature, both forms remained unchanged for more than a month; after 6 months, only a small amount of anhydride formation was found to have occurred. The second group of figures in Table II shows that only a small amount of anhydride formation occurs during the melting of the crystals.

The rotations of the free acids and their salts are given in Table IV. The measurements were made at 21–22° in a 2 dm. tube, with the mercury green line (λ 5461 Å.). The difference in the specific rotations of the two sodium salts is the usual effect of changing the concentration.

The biological activity of the two optically active forms was tested with lactic acid dehydrogenase prepared from muscle by the method of von Szent-Györgyi (11). When the *l*(+) form was added to the enzyme and methylene blue in an evacuated Thunberg vessel, the dye was quickly decolorized. The *d*(−) form, on the other hand, was quite inactive, giving a longer decoloration time than the enzyme alone. We are indebted to the kindness of Mr. H. F. Schott for this examination of the two forms of lactic acid prepared, and for his active interest and many helpful suggestions throughout the course of this work.

SUMMARY

1. Two methods are described for obtaining optically active lactic acid (both isomers) from a commercial aqueous syrup.

2. A method is described of preparing the active isomers in a crystalline state, free of water, anhydride, and lactide.

3. Some of the properties of the crystalline acids are described. The following physical constants are given: melting points, *l*(+)-lactic acid, 52.8°; *d*(−)-lactic acid, 52.8°; *dl*-lactic acid, 16.8°; the acid dissociation constant of the three forms at 25° is pK = 3.81 ± 0.02.

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